



SEQUENCE LISTING

<110> THASTRUP, Ole
TULLIN, Soren
POULSEN, Lars K
BJORN, Sara P

<120> Novel Fluorescent Proteins

<130> 3759-0106P

<140> 09/619,310

<141> 2000-07-19

<150> US 08/819,612

<151> 1997-03-17

<150> PCT/DK96/00051

<151> 1996-01-31

<150> DK 1065/95

<151> 1995-09-22

<160> 23

<170> PatentIn version 3.1

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<223> GFP2 primer directed to A. victoria

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OCT 22 2002

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<223> 5' PCR primer incorporating the Y66H substitution responsible for changing green fluorescence into blue fluorescence

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<223> 3' PCR primer incorporating the Y66H substitution responsible for changing green fluorescence into blue fluorescence

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Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe		
35 40 45		
att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act		193
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr		
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Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
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aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag 289
 Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
 80 85 90

gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt gct 337
 Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
 95 100 105 110

gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa 385
 Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
 115 120 125

ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa 433
 Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu
 130 135 140

tac aac tat aac tca cat aat gta tac atc atg gca gac aaa cca aag 481
 Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys
 145 150 155

aat gga atc aaa gtt aac ttc aaa att aga cac aac att aaa gat gga 529
 Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly
 160 165 170

agc gtt caa tta gca gac cat tat caa caa aat act cca att ggc gat 577
 Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
 175 180 185 190

ggc cct gtc ctt tta cca gac aac cat tac ctg tcc acg caa tct gcc 625
 Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
 195 200 205

ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt gag 673
 Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu
 210 215 220

ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa 721
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Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn
130 135 140


Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

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ctt	gtt	gaa	tta	gat	ggc	gat	gtt	aat	ggg	caa	aaa	ttc	tcc	gtt	agt	97
Leu	Val	Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	Gln	Lys	Phe	Ser	Val	Ser	
15				20				25						30		
gga	gag	ggt	gaa	ggt	gat	gca	aca	tac	gga	aaa	ctt	acc	ctt	aaa	ttt	145
Gly	Glu	Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	
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Ile	Cys	Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Pro	Trp	Pro	Thr	Leu	Val	Thr
50				55					60							
act	ctc	tct	cat	ggt	gtt	caa	tgc	ttt	tct	aga	tac	cca	gat	cat	atg	241
Thr	Leu	Ser	His	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	
65				70					75							
aaa	cag	cat	gac	ttt	ttc	aag	agt	gcc	atg	ccc	gaa	ggt	tat	gta	cag	289
Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	
80				85				90								
gaa	aga	act	ata	ttt	tac	aaa	gat	gac	ggg	aac	tac	aag	aca	cgt	gct	337
Glu	Arg	Thr	Ile	Phe	Tyr	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	
95				100				105			110					
gaa	gtc	aag	ttt	gaa	ggt	gat	acc	ctt	gtt	aat	aga	atc	gag	tta	aaa	385
Glu	Val	Lys	Phe	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys		
115				120					125							
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Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Met	Glu	
130				135					140							
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Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Pro	Lys	
145				150					155							
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Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Lys	Asp	Gly	
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Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala				
195	200	205		
ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt gag				673
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu				
210	215	220		
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Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys				
225	230	235		
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Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys				
35	40	45		
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu				
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Ser His Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln				
65	70	75	80	
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg				
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Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val				
100	105	110		
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile				
115	120	125		
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn				

130

135

140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly
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Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
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Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu Phe Val
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gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt 145
Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
35 40 45

att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 193
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
50 55 60

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Thr Leu Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met

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aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag			289
Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln			
80	85	90	
gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt gct			337
Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala			
95	100	105	110
gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa			385
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys			
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ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa			433
Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu			
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160	165	170	
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Asp Lys Pro Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn			
175	180	185	190
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195	200	205	
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210	215	220	
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Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met			
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Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
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Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
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His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
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Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly
145 150 155 160

Met Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys
165 170 175

Pro Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys
180 185 190

Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile
195 200 205

Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln
210 215 220

Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu
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Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu
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Tyr Lys

<210> 21
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Leu Val Glu Leu Asp Gly Asp Val Asn Gly Gln Lys Phe Ser Val Ser
15 20 25 30

gga gag ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt 145
Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
35 40 45

att tgc act act ggg aag cta cct gtt cca tgg cca acg ctt gtc act 193
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
50 55 60

act ctc act tat ggt gtt caa tgc ttt tct aga tac cca gat cat atg 241
Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
65 70 75

aaa cag cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag 289
Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
80 85 90

gaa aga act ata ttt tac aaa gat gac ggg aac tac aag aca cgt gct 337
Glu Arg Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
95 100 105 110

gaa gtc aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa 385
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
115 120 125

ggt att gat ttt aaa gaa gat gga aac att ctt gga cac aaa atg gaa 433

Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu		
130	135	140
tac aat tat aac tca cat aat gta tac atc atg gca gac aaa cca aag		481
Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys		
145	150	155
aat ggc atc aaa gtt aac ttc aaa att aga cac aac att aaa gat gga		529
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly		
160	165	170
agc gtt caa tta gca gac cat tat caa caa aat act cca att ggc gat		577
Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp		
175	180	185
ggc cct gtc ctt tta cca gac aac cat tac ctg tcc acg caa tct gcc		625
Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala		
195	200	205
ctt tcc aaa gat ccc aac gaa aag aga gat cac atg atc ctt ctt gag		673
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu		
210	215	220
ttt gta aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa		721
Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys		
225	230	235
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 20 25 30

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 35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
 50 55 60

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85 90 95

Thr Ile Phe Tyr Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Met Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Pro Lys Asn Gly
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Lys Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205


Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225 230 235

<210> 23
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<212> DNA
<213> Artificial Sequence

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<223> DNA sequence at the lacZ-promoter GFP fusion point

<400> 23
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14